The role of pharmacogenetically-variable cytochrome P450 enzymes in drug abuse and dependence

Lisa A Howard[1], Edward M Sellers[1,2,3] & Rachel F Tyndale[†1-3]

[†]Author for correspondence Dept. of Pharmacology, 1 King's College Circle, University of Toronto, Toronto, Canada, M5S 1A8 Department of Pharmacology[1], Department of Psychiatry[2], Centre for Addiction and Mental Health[3], University of Toronto, Toronto, Ontario, Canada, M5S 1A8 Tel.: +1 416 978 6374; Fax: +1 416 978 6395; E-mail: r.tyndale@utoronto.ca

The risk of drug dependence is determined by the interaction of drug, individual and environment. 'Pharmacogenetics' is the study of the influence of heredity on the response to drugs and their fate in the body; these studies aim to improve the understanding of inter-individual variability in drug response. The authors have applied this research approach to the study of drug metabolism and dependence. Specifically the interaction of genetically variable hepatic cytochrome P450 (CYP) enzymes and their effect on self-administration of drugs has been examined. Many drugs of abuse are substrates (e.g., amphetamines, codeine, nicotine) or inhibitors (e.g., (-)-cocaine) of polymorphic CYPs. Drug metabolism by genetically polymorphic enzymes can have significant clinical implications relating to drug toxicity, therapeutic failure, drug-drug interactions, disease susceptibility and abuse liability. There is good evidence that drug metabolism by genetically variable CYPs can influence the risk of drug dependence, the amount of drug consumed by dependent individuals and some of the toxicities associated with drug-taking behavior. It is anticipated that pharmacogenetics will be used to identify individuals at a greater risk for specific drug dependencies, provide information that can lead to novel treatment and prevention approaches as well as provide guidance for individualization of treatment choice.

Keywords: codeine, cytochrome P450 enzymes, dextromethorphan, drugs of abuse, ethanol, flunitrazepam, genetic risk factor, smoking, nicotine, N-methylamphetamine, therapeutic intervention

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CYP polymorphisms

CYPs are mixed function oxidases that are predominantly expressed in the liver and biotransform drugs, endogenous compounds, dietary constituents and environmental toxins [1]. The mammalian superfamily consists of > 100 genes [2]. Genetic variation in drug metabolism was first detected in humans by the observance of adverse reactions after normal drug doses. It has been estimated that genetic factors could account for ~ 20–40% of interindividual differences in drug metabolism and response; since the majority of drug-metabolizing CYPs are genetically polymorphic, these enzymes will substantially contribute to this variation [3]. The authors have shown that polymorphic metabolism by these CYPs can change self-administration of drugs, altering both the risk for dependence and the amount consumed.

It has become increasingly evident that genetic polymorphisms are a major determinant of the interindividual variation observed in CYP expression and activity. This genetic variation may result in the absence of a gene product, increased, reduced or altered enzyme activity as well as altered regulation and inducibility of the enzyme. A pharmacogenetic polymorphism refers to a phenotypical subgroup in a population with a frequency > 1%. Individuals can be designated into the following phenotypic groups: poor (PM), slow (SM), extensive (EM), and fast (FM) metabolizers, which consist of people with 0, 0 or 1, 1 or 2, and 2+ active copies of the CYP gene. CYPs are designated by a family number, a subfamily letter, a number of the particular family member and a number for each variant (e.g., *CYP2A6*2*). A genotype consists of the two alleles in each person. With respect to nomenclature, generally the wild type functional allele is denoted as the *1 allele and subsequent allelic variants are denoted as *2, *3, *4, etc. based on order of discovery (allele nomenclature published at [201]). Gene duplications are indicated by the allele which is duplicated and the number of copies identified (e.g., *CYP2D6*2x2*). Allelic variants can be generated by:

- gene deletions
- gene conversions with related pseudogenes
- single base substitutions causing frameshift missense, nonsense or splice-site mutations [3]

An extensive summary of polymorphic *CYP* alleles can be found at [201].

Behavioral phenomenon of drug dependence

Drug abuse involves a maladaptive pattern of drug use, such that significant adverse consequences occur (e.g., failure to fulfill major role obligations

and interpersonal problems) [4]. Drug dependence is a more serious condition characterized by compulsive drug-taking behavior despite significant drug-related problems and involves the development of tolerance and withdrawal [4]. Individuals of varied metabolic genotypes may experience different responses to some drugs of abuse whose use is governed to a great extent by their pharmacological effect(s). Potential drugs of dependence must exhibit specific characteristics, such as acting as a reinforcer (i.e. once taken, the probability increases that the drug will be taken again). Reinforcement manifests as having either positive rewarding effects (e.g., subjective euphoria) or removing punishing experiences (e.g., alleviating depression during cocaine withdrawal). If a drug has 'punishing' or averse qualities, such as with nicotine, the increased levels of the parent drug in PM individuals (due to decreased rates of metabolism) might decrease the likelihood of seeking successive doses, thereby reducing their chance of learning the addictive behavior. Alternatively, increased plasma drug levels might increase the risk of abuse and dependence by increasing the threshold for reinforcement through the development of tolerance. Successively higher doses are thus needed to achieve the same pharmacological effect.

Drugs with aversive properties are less likely to be abused unless tolerance to the unpleasant effects occurs. There are several other features of a drug that influence the likelihood of its abuse, such as its rate of absorption and onset of effect. Highly lipid soluble drugs are likely to reach the brain more rapidly when compared to drugs which are more water soluble. The increased speed of delivery to their sites of action in the brain increases the relative likelihood of lipid soluble drugs being used illicitly [5]. For example, diazepam is more lipid soluble and rapidly absorbed than oxazepam making diazepam preferred by benzodiazepine abusers, recovering heroine addicts and alcohol abusers [5]. A drug's metabolic profile is another key factor influencing its abuse liability. For example, heroine has negligible pharmacological activity itself and its addictive potential is directly dependent on its conversion to 6-mono-acetylmorphine, which is a potent opioid capable of producing euphoric effects [6]. Since the pharmacodyamic effects associated with drugs could be mediated by either the parent drug or its metabolite, the impact of rapid or slow metabolism on the initiation of drug dependence will depend on whether the metabolism of a particular drug results in pharmacological inactivation of an active drug or activation of a prodrug. Altered drug metabolism may also affect the amount and pattern of drug abuse. For example, decreased drug metabolism may result in a reduction of the dose used by an individual as the drug remains in the body for a longer time increasing the time to withdrawal.

Although factors affecting drug dependence are complex, reliable and validated measures of the behavior exist that facilitate its accurate and interpretable investigation. Methodological techniques to quantify the frequency of drug-taking, the objective and subjective consequences of drug use, the physiologic effects of a drug and the socio-economic impact of drug abuse allow the consequences of variation in drug disposition on drug dependence and abuse to be examined.

Impact of *CYP* genetic polymorphisms on dependence risk

'Pharmacogenetics' is the study of inherited differences in responses to drugs; this field has progressed towards developing tests, which will be used to predict which patients are likely to benefit from a medicine, which are likely to suffer a toxic side effect and which may be therapeutically under- or over-dosed [7]. Of clinical relevance is the application of pharmacogenetics to the study of drug abuse and dependence. Many drugs of abuse, including nicotine, codeine, (-)cocaine and several amphetamine analogues are known substrates and/or inhibitors of CYP enzymes [8]. Variation in pharmacokinetics for some of these drugs, due to genetically variable polymorphic enzyme activity, may be large enough to outweigh pharmacodynamic sources of variation in drug response [9]. Genetic differences in the metabolism of drugs of abuse may partially explain why some individuals are more susceptible to drug abuse than others. Moreover, interethnic differences in the abuse patterns of certain drugs (e.g., smoked amphetamine) may be partly due to underlying variation in drug metabolism [9].

The consequence of absent or altered CYP activity for any particular drug will depend on the relative activity of the parent drug and its metabolite(s) as well as their respective half-lives. Metabolite pharmacology can be qualitatively similar (e.g., codeine to morphine) or differ from the parent drug (e.g., dextromethorphan to dextrorphan). The behavioral consequences of *CYP* polymorphisms should relate to genotype and

absolute CYP activity. Therefore, individuals with a deficient CYP activity (PMs) will have a decreased probability of becoming dependent (or slower acquisition or altered pattern of use) on a drug converted to an active metabolite capable of maintaining drug-taking (e.g., dextromethorphan), while EMs should have an increased probability of dependent behavior (e.g., more rapid onset, use of higher doses). Conversely, PMs should experience a greater risk of toxicity to a drug that is detoxified by a polymorphic CYP, and EMs a lesser toxicity. Other toxicities may be associated with the drug taking behavior, independent of the specific drug of abuse. For example, as in the case of tobacco, CYP2A6 EMs may be at lower risk for toxicity from nicotine, due to more rapid inactivation but be at greater risk from toxicities associated with a greater exposure and activation of tobacco pro-carcinogens. Individuals and populations exist that lack some of these CYPs (e.g., PMs), the enzymes can be inhibited without important clinical risk of general toxicity (keeping alternative drug-interactions in mind). This offers the opportunity to develop new therapeutic approaches for drug dependence. These studies may contribute to the understanding of prevention and treatment of dependence on CYP substrates such as oral opiates, nicotine, alcohol, methamphetamine and benzodiazepines.

CYP2A6

From numerous studies among various ethnic groups, it has been estimated that familial inheritance is responsible for at least 50% (up to 84%) of the risk for tobacco dependence [10-12]. The maintenance and level of tobacco smoking has been predicted to have an even greater proportion of risk (70–86%) that is related genetics [10,11]. Nicotine is the tobacco constituent that is responsible for establishing and maintaining tobacco dependence [13]. A positive correlation exists between breath carbon monoxide levels (an indicator of smoke inhalation which is used to verify cigarette number estimates) and plasma nicotine levels, while a negative correlation occurs between plasma nicotine levels and the craving for cigarettes [14]. These observations support the hypothesis that the subjective measure of 'urge to smoke' is directly associated with nicotine seeking in tobacco-dependent smokers. Examining the variability in nicotine inactivation is clinically relevant, since smokers adjust their smoking pattern and intensity to maintain desirable plasma or CNS nicotine levels [15]. The variation in rates of nicotine metabolism could influence individual plasma and brain nicotine levels, nicotine accumulation, smoking patterns and attempts to quit [16].

In humans, ~ 70–80% of nicotine is metabolized to the inactive product cotinine [17]. The recently identified genetically polymorphic CYP2A6 enzyme is responsible for the majority of metabolic conversion/inactivation of nicotine to cotinine [18]. Of the numerous CYP isozymes, CYP2A6 shows among the greatest interindividual variation in activity, mRNA and immunoreactivity levels [19-21]. Large variation is seen in human liver *in vitro* metabolism by CYP2A6, ranging from 20- to 2000-fold with various selective CYP2A6 substrates [18,21]. This variability is primarily due to genetic variation in the *CYP2A6* gene locus. Several genetic variants of *CYP2A6* have been identified:

- *CYP2A6*1A* and **1B* that have normal activity [21,22]
- *CYP2A6*1X2* exhibits increased activity (at least three *CYP2A6* allelic copies) [23]
- *CYP2A6*2, 3, 4* and *5* are associated with absent activity [21,24-26]

Data from a recent study conducted by the authors indicated a decrease and increase in nicotine metabolism in those with null or duplicated variants, respectively [27]. Three recently identified alleles, *CYP2A6*6, *7* and **9*, exhibit reduced activity compared to the wild type allele when tested *in vitro* [28-30]. The effect of *CYP2A6*7* on *in vivo* nicotine and coumarin metabolism was examined. Preliminary assessment indicates that the *CYP2A6*7* allele resulted in a decreased activity towards nicotine, the *CYP2A6*8* was fully functional, while the allele with both the *CYP2A6*7* and **8* substitutions (named *CYP2A6*10*) appeared to have dramatically reduced function [31].

Individuals who converted nicotine to an inactive product more slowly (CYP2A6 SMs) [18], were postulated to be less likely to become dependent smokers for two reasons. First they would have greater levels of nicotine when learning to smoke, thereby augmenting nicotine's noxious, aversive effects; in addition, CYP2A6 SMs (one or two inactive alleles) would experience a slower development of withdrawal and tolerance. In their original study, tobacco-dependent subjects with inactive alleles were found to be at a lower risk of becoming tobacco dependent than those with two functional copies of the gene (*CYP2A6*1/*1* or EMs) [32]; however,

there were issues with the genotyping assays [24]. Data from subsequent follow up studies indicated that among Caucasians recruited and genotyped for *CYP2A6*2* and **4*, significantly more of those tobacco non-dependent than tobacco dependent were SMs. Similarly, we found that more non-dependents than regular smokers were SMs among Chinese subjects (i.e., carried at least one inactive *CYP2A6*4* allele) (Tyndale and Sellers, unpublished observations).

The authors also predicted that those CYP2A6 SMs who learned to be regular smokers would require fewer cigarettes/day to maintain their nicotine levels and dependence. It was found that tobacco-dependent CYP2A6 SMs smoked fewer cigarettes than tobacco-dependent smokers who were EMs (129 *vs.* 159 cigarettes/week, t-test $p < 0.02$) [32]. These findings have subsequently been replicated in a broader investigation [23]. Smokers ($n = 400$) were extensively characterized for smoking habits such as reported cigarettes/day, maximal time period of smoking and DSM-IV and Fagerstrom dependence evaluations. Objective measures of smoking, including levels of nicotine and cotinine in urine and plasma as well as breath carbon monoxide levels, were also collected. Among 296 Caucasian smokers, SMs smoked fewer cigarettes/day compared to EMs (also evident from lower carbon monoxide levels) to maintain equal plasma nicotine levels [23]. Consistent with alterations in the metabolic inactivation of nicotine, several indices of smoking revealed that subjects who were:

- SMs (≤ 1 active allele)
- EMs (2 active alleles)
- FMs (≥ 3 active alleles)

displayed significantly different smoking patterns and intensities. In particular, subjects with the *CYP2A6* duplication allele (FMs) smoked each cigarette more intensely as indicated by a doubling of the CO/cigarette ratio and higher plasma nicotine/cigarette compared to wild type homozygotes (EMs) or those with at least one null allele (SMs) [23]. It appears that individuals with duplicated *CYP2A6* alleles increase their smoking by increasing the intensity of inhalation of each cigarette, while those with null *CYP2A6* alleles decrease their smoking levels by smoking fewer cigarettes/day [23]. These data demonstrate a role for *CYP2A6* gene variants in affecting smoking patterns.

The observations of other investigators also support a role of CYP2A6 in the determination of aspects of smoking. One study has shown that individuals who carried a *CYP2A6*2* allele initiated smoking later in life (17.5–20.5 years), smoked for a shorter time period (9 years) and showed an increased incidence of quitting (relative risk of 1.75, which increased with the number of *CYP2A6*2* alleles carried) [33]. Among Japanese individuals, a significant correlation was observed between *CYP2A6* genotype and cotinine concentrations. In addition, there was a declining frequency of PMs homozygous for *CYP2A6*4*, as the number of cigarettes/day increased (9.7% of light, 4.3% of intermediate and 3.0% of heavy smokers) [34]. A study in Chinese showed that the frequency of SMs (*CYP2A6*1/*4* and **4/*4* individuals) was greater in those smoking < 25 cigarettes/day (18.7%) relative to those smoking ≥ 25 cigarettes/day (12.5%) but no effect was seen on risk [35]. Some data support the role for *CYP2A*6 genetic variation in smoking, both in risk for dependence and amount smoked, while others do not. Likely contributors to differing outcomes for *CYP2A6* genetic association studies include:

- different definitions of 'smokers' (i.e., ever *vs.* never, former, >100 cigarettes lifetime, pack-years, dependence), 'smoking behavior' (i.e., initiation, maintenance, quitting, cessation, relapse) and ethnicity
- inclusion of functionless polymorphisms
- methodological and statistical power issues

The evidence that these discrepancies are serious issues is demonstrated by the observation that 11% of a recent sample of individuals from our database who had ever smoked > 100 cigarettes (a commonly used definition in studies) were not tobacco dependent and had allele frequencies similar to non-smokers. Outcomes may differ depending on definitions of smoking. Similarly, the ethnic background of a studied individual should be accounted for in any analysis since profoundly differing allele frequencies among ethnic groups may confound interpretation of studies with mixed ethnicity. Subsequently, CYP2A6 inhibition studies were performed to directly assess the effects of inhibited CYP2A6 on smoking behavior. Consistent with the observations of decreased smoking among individuals with genetically lower amounts of CYP2A6, inhibiting the enzyme in smokers *in vivo*, with or without additional nicotine, resulted in decreased smoking (e.g., increased interval between cigarettes) [37].

CYP2D6

CYP2D6 metabolizes many drugs (current estimate ~ 45) and is a particularly interesting example of the potential role for a polymorphic enzyme in drug abuse. CYP2D6 interacts with numerous psychoactive chemicals including antidepressants, antipsychotics, analgesics and CNS stimulants. In addition, it has been shown that CYP2D6 is expressed in the human brain and may alter the inactivation or production of drug and/or metabolites at their site of action [37]. Hence, CYP2D6 may have a role in modulating central functional pathways that are involved in drug-reinforced behavior and/or neurotoxicity [9]. The highly polymorphic nature of *CYP2D6* (~ 60 allelic variants identified) manifests as the extensive (wild type *CYP2D6*1* allele); intermediate (reduced activity e.g., *CYP2D6*2*, **10* and **17* alleles); inactive (e.g., *CYP2D6*3*, **4* and **5* alleles) and ultrarapid (*CYP2D6*2XN* alleles) metabolizer phenotypes and reflects the high interindividual and interethnic differences in the metabolism of CYP2D6 substrates.

Dextromethorphan is an opioid agonist that is essentially devoid of analgesic effects but commonly used for its potent antitussive actions in anticough medicinal formulations. There are some reports of its abuse at high doses, due to its ability to produce sedation, agitation, dissociative sensations and visual hallucinations [38,39]. Dextromethorphan is metabolized by CYP2D6 to dextrorphan, which is thought to mediate the abuse potential of dextromethorphan since animals recognize the metabolite as a discriminative stimulus and generalize it to phencyclidine [40]. *In vivo* kinetic studies show profound differences in dextromethorphan metabolism between individuals that exhibit low and high CYP2D6 activity. PMs exhibit a significantly longer $t_{1/2}$ of dextromethorphan and a lower area under the plasma concentration-time curve (AUC) of its metabolite dextrorphan compared to EMs [41]. In the first study comparing the subjective and psychomotor effects of dextromethorphan in EMs and PMs, it was found that PMs reported greater effects with measures of sedation (ARCI pentobarbital-chlorpromazine-alcohol scale) and dysphoria (ARCI lysergic acid diethylamide scale) as well as with visual analog scales (VAS) of 'bad' drug effects compared to EMs. In contrast, EMs described greater effects on the Cole/ARCI abuse potential scale and the VAS scales of 'good' drug effects and drug 'liking'. There was a high correlation between dextromethorphan concentration and VAS ratings of 'bad' drug effects. Moreover, the AUC plasma dextromethorphan values correlated with VAS for drug strength and sedation measures while the AUC plasma dextrorphan values correlated with abuse potential and drug 'liking' scales [42]. Hence it is evident that qualitative differences in the subjective effect profiles of dextromethorphan exists between EMs and PMs and the findings suggest that EMs may have a greater risk of abuse for this drug than PMs since EMs experienced positive subjective effects while PMs appear to be at greater risk of adverse effects.

Codeine is an opioid therapeutically used for its analgesic and antitussive properties and is also a highly misused drug. Although a minor metabolic pathway, codeine O-demethylation by CYP2D6 to morphine is the clinically relevant pathway of metabolism as morphine and its metabolites are more potently analgesic than codeine. In addition to pain-relief, the subjective effects of opioids (i.e., elation, euphoria, 'liking' etc.) are mediated through the μ opioid receptors [43]. In an epidemiological study, the authors tested whether the failure to activate codeine was a protective factor in oral opiate dependence. It was found that PMs were significantly less likely to become oral opiate dependent ($p < 0.05$, estimated odds ratio > 7), suggesting that PMs do not experience sufficient reinforcing effects to initiate and maintain oral opiate use [44]. Interestingly, no ultrarapid metabolizers were found among the codeine-dependent individuals, which may indicate that the rapid conversion of codeine to morphine increases side effects and confers protection from oral opiate dependence.

The CYP2D6 substrate N-methylamphetamine (MAMP), also known as methamphetamine, is abused in many parts of the world but especially in Asian countries, including China, Japan and Taiwan [45,46]. CYP2D6 mediates the p-hydroxylation of MAMP to a less active metabolite [47,48]. The expected increased levels of the more centrally active parent amphetamines may alter their abuse liability in those with inactive or reduced activity alleles. Kinetic studies have demonstrated that PMs were more sensitive to methamphetamine-induced effects as measured by Cole/ARCI sedation-motor, stimulation-motor, stimulation-euphoria scales, ARCI and MGB scales as well as VAS rating of 'good effects' [50]. Asian populations have a high frequency of the reduced activity *CYP2D6*10* allele which may partly explain the lower mean activity of CYP2D6 in Asian EMs compared to

their Caucasian counterparts. The decreased activity of *CYP2D6*10* has been shown to be clinically relevant to the metabolism of many psychotropic medications (e.g., venlafaxine [50], nortriptyline [51] and trazodone [52]). The authors' findings of the profound decreases in amphetamine metabolism by *CYP2D6*10 in vitro* compared to wild type CYP2D6 imply that *CYP2D6*10/*10* individuals may display altered susceptibility to developing dependence to these drugs [53].

CYP2C19

Flunitrazepam is the benzodiazepine with the highest abuse liability; this drug is reported to be the most widely abused benzodiazepine among opioid abusers worldwide [54]. *In vitro* and *in vivo* studies have established that CYP2C19 mediates part of the conversion of flunitrazepam to N-desmethyl-flunitrazepam and 3-OH-flunitrazepam [55]. The impact of the *CYP2C19* polymorphism on abuse properties of diazepam and flunitrazepam has been examined. A kinetic pilot study of CYP2C19 by phenotype suggested that PMs had higher plasma flunitrazepam concentrations, experienced more sedation and 'spacey feeling' and exhibited greater psychomotor impairment compared to EMs, suggesting that CYP2C19 may play a clinically relevant role in the metabolism of flunitrazepam *in vivo* [56].

Benzodiazepines appear to be metabolized at a slower rate in Asians compared to Caucasians [57,58]. Studies indicate that Chinese patients living in Hong Kong were treated with substantially smaller amounts of benzodiazepines compared to Caucasians [57]. The high frequency of inactive *CYP2C19* alleles in Asians [59] implicate *CYP2C19* genotype as a determinant of inter-ethnic differences in response to benzodiazepines. Chinese are reported to be more sensitive than Caucasians to benzodiazepines [60] and flunitrazepam appears to be a preferred drug of abuse among Taiwanese-Chinese (Dr Lien Wen Su, Taipei City Psychiatric Centre, Taiwan, personal communication).

To investigate the role of CYP2C19 in flunitrazepam's pharmacodynamics effects, 2C19-mediated hydroxylation of flunitrazepam was inhibited using omeprazole and a trend towards decreased psychomotor performance (an objective measure of a flunitrazepam effect) with decreasing CYP2C19 activity was found ($p = 0.11$) (Gafni *et al.* unpublished observations). The data suggest that individuals with lower CYP2C19 activity may experience greater psychomotor impairment and sedation with flunitrazepam. This greater responsiveness might explain the increased sensitivity to benzodiazepines observed in Asians and potentially the higher misuse of benzodiazepines in these populations. There were several limitations in the subjective responses that may have influenced the results such as:

- intra- and interindividual variability in the dose-response relationship
- mood states
- personality
- interpretation or understanding of the descriptors used in measuring subjective effects

It remains to be resolved whether flunitrazepam metabolism, clinical effects and abuse potential are influenced by the *CYP2C19* polymorphism.

CYP2E1

Although alcohol dehydrogenase is the major contributor to ethanol metabolism [61], CYP2E1 also contributes to ethanol metabolism and is the only ethanol-metabolizing enzyme that is induced by ethanol consumption [62]. Hence, CYP2E1's induction by chronic ethanol intake may contribute to the adaptive increase in ethanol metabolism observed in alcoholics [62]. Marked interindividual variation in CYP2E1 activity exists, with 12- to 30-fold variation in hepatic CYP2E1 levels reported in humans [63]. Genetic variation may explain the marked interindividual variability in basal and ethanol-induced CYP2E1 activity [63]. The coding region of *CYP2E1* appears to be well conserved [1], but some recent investigations have suggested that polymorphisms exist which alter CYP2E1 inducibility. In a French study, 20% of alcoholic patients exhibited a low CYP2E1 inducibility phenotype that was measured using the probe drug chlorzoxazone [64]. Notably, this study showed that CYP2E1 inducibility correlated to the extent of autoantibodies formed against a CYP2E1-generated metabolite of ethanol [64]. The most well studied polymorphic sites of *CYP2E1* are located in the regulatory region of the gene (*CYP2E1*5B* or c2 allelic variant) adjacent to a putative HNF1α- element and in intron 6 (*CYP2E1*6* or C variant). These variants appear to influence CYP2E1 inducibility since a study of alcoholic patients showed that ethanol-induced subjects who were wild type homozygotes had a significantly greater level of CYP2E1-mediated chlorzoxazone metabolism than the heterozygotes carrying either variant allele [65]. Moreover, *CYP2E1*5B* was associated with greater ethanol

elimination rates [66] even after accounting for alcohol dehydrogenase (ADH) gene variants [67]. However, other studies of the *CYP2E1*5B* variant failed to find a correlation with the *in vivo* metabolism of chlorzoxazone [68]. In addition, there are conflicting reports on the impact of these variants on the risk of alcohol dependence [66,69].

Recently the *CYP2E1*1D* allele was identified which has a repeat sequence in the 5′-flanking region of the gene and appears to exhibit increased inducibility compared to the wild type *CYP2E1*1C* allele [70]. This variant allele was associated with a 2.5-fold greater CYP2E1 activity in African American females only under conditions that are known to induce CYP2E1. Although recent studies did not find an association between *CYP2E1*1D* and the incidence of alcoholism or CYP2E1 inducibility, it may be important to consider several issues when interpreting these findings. Itoga and associates compared the frequencies of *CYP2E1*1D* (referred to as the A4 (372 base pair allelic variant) in three groups of Japanese individuals:

- 79 non-drinkers and moderate drinkers
- 113 heavy drinkers
- 202 alcoholics

No significant differences were identified between the groups with respect to allele and genotype frequencies [71]. Interestingly, there was an increasing trend for the homozygote *CYP2E1*1D/*1D* frequency in the alcoholic group compared to the control groups while the frequency of heterozygotes showed a decreasing trend suggesting that the effect of the *CYP2E1*1D* polymorphism on alcohol dependence may exist and show a gene-dose effect. Since only 12 homozygotes were compared in this study, perhaps a larger study would allow the examination of the impact of *CYP2E1*1D* homozygosity on the risk for alcoholism. Findings from a study in French Caucasians found no significant difference in *CYP2E1*1D* frequency between 103 controls (1.46%) and 246 alcoholics (1.63%) [71]; however, the extremely low frequency of this allele in Caucasians observed by this group and others [72] indicates the need for much larger studies.

Plee-Gautier and colleagues examined the impact of *CYP2E1*1D* on *in vivo* CYP2E1 inducibility by comparing the metabolism of chlorzoxazone between two heterozygotes and eight homozygotes pre- and 12 hours post-administration of 0.8 g/kg of ethanol and found no significant difference in CYP2E1 inducibility between the two groups [71]. Although this finding casts doubt on the functional relevance of *CYP2E1*1D*, most *in vivo* CYP2E1 studies in humans that have shown the induction of chlorzoxazone metabolism by chronic ethanol exposure (i.e., currently drinking alcoholics were compared to either withdrawn alcoholics or non-drinking controls) [63,65]. Hence, it remains to be determined whether the *CYP2E1*1D* variant, that putatively acts to enhance transcriptional induction of CYP2E1, is associated with enhanced CYP2E1 induction during chronic ethanol consumption and whether this alters the risk for dependence or the amount of ethanol consumed by those dependent.

To further examine the clinical implications of having the *CYP2E1*1D* variant, its influence on the risk for alcoholism in different ethnic groups is being assessed. The sample of alcoholics will be stratified into abusers and DSM-IV dependents will be stratified and the influence of *CYP2E1*1D* on the amount of alcohol consumed and/or the risk for dependence will be investigated. Of note, it has been found that there is a greater frequency of *CYP2E1*1D* in African Americans compared to Caucasians [70,72,73]. African Americans also appear to be more susceptible to alcoholism even after adjusting for education and socio-economic status [74,75]. As was postulated with the ADH2*3 polymorphism (a variant that is associated with greater ethanol elimination rate), which is also at a higher frequency in African Americans than Caucasians [76], the *CYP2E1*1D* may contribute to the greater susceptibility of African Americans to alcoholism. In addition, genetic variation in dopamine receptors, serotonin transporters and ethanol-metabolizing dehydrogenases has also been hypothesized to contribute to risk for alcoholism [77].

An estimated 80–95% of alcoholics smoke regularly [10,78,79]. Alcoholics who smoke also drink higher quantities, drink more frequently, report higher urges to drink and have more difficulty quitting drinking than those that do not smoke [80,81]. Twin studies indicate that for equal ethanol consumption, heavy smokers have higher ethanol elimination rates (~ 1.9-fold) than non-smokers [82]. In humans there is some suggestion that cigarette smoking can enhance CYP2E1 activity [83]; however, not all studies agree [84]. Tobacco smoke can increase CYP2E1 in animals [85] and it has been shown that lower doses of nicotine can increase CYP2E1 activity in rats [87]. Of particular note,

genetic linkage studies have identified markers that associate with alcoholism [87,88] as well as alcoholism and smoking [89] near the *CYP2E1* loci (10q24.3) [87]). Both ethanol and nicotine can induce CYP2E1 activity, and it is hypothesized that ethanol or nicotine exposure may increase CYP2E1-mediated ethanol elimination thereby providing an impetus for increased ethanol consumption. Individuals with a greater inducibility of CYP2E1 may be at a greater risk to higher ethanol intake when exposed to nicotine during cigarette smoking. Hence it would be of interest to ascertain whether there is a higher incidence of the *CYP2E1*1D* variant among alcoholic smokers compared to alcoholic non-smokers. Individuals with higher induced CYP2E1 levels may be more susceptible to the development of greater ethanol consumption, leading to a greater risk of ethanol abuse and dependence as well as to ethanol-related diseases.

Diagnostic and prognostic implications

Advances in the understanding of the genetics and neurobiology of drug addiction may have dramatic diagnostic and prognostic implications. It is possible that in the future a patient with a health problem, such as a drug dependency, will undergo a series of tests (e.g., genetic tests, brain-imaging scans) that can be used to define the disorder and aid in choosing the most appropriate course of treatment. Of the polymorphisms that affect the efficacy of current drugs (e.g., gene variants encoding drug receptors, drug transporters and cell signaling pathways), investigators have proposed that the most diagnostically usable pharmacogenetic variants are those in genes contributing to drug metabolism and disposition [7]. Hence, it may be feasible to identify and educate individuals that have a relatively higher vulnerability to the pharmacodynamic effects of a specific drug, those for whom even brief exposure to a drug of abuse pose a substantial risk for addiction thereby targeting prevention more effectively.

In addition to influencing the risk of drug dependence, genetic polymorphisms are likely to contribute to pathogenesis of diseases related to the misuse of these drugs. Both CYP2A6 and CYP2E1 not only metabolize drugs but also mediate the bioactivation of several toxins to their reactive intermediates including the tobacco procarcinogens ((4-methyl-nitrosamino)-1-(3-pyridyl)-1-butanone or (NNK)) and nitrosodimethylamine, respectively [90,91]. A preliminary analysis of the epidemiological data suggest that individuals with defective *CYP2A6* allele(s) have lower K_i-ras oncogene mutations (codon 12) in biopsies from lung tumors and lower incidences of lymphomas (Tyndale and Tsao, unpublished data), which is supported by other investigations of *CYP2A6* variants and cancer incidence. Similarly, the presence of defective *CYP2E1* allelic variants appear to confer protection against the incidence of lung cancer [92], which may be specific to histologic types; however, other studies have failed to find this association [93]. CYP2D6 has been implicated in the pathogenesis of many disease states including Parkinson's disease, Alzheimer's disease, epilepsy and various forms of cancer [94-98]. Although the exact role of CYP2D6 remains unclear, several studies have indicated different susceptibility for individuals of different *CYP2D6* genotypes. Genotyping for these polymorphisms may enhance the understanding of the physiological factors underlying the pathogenesis of diseases, particularly pathologic conditions related to protoxicant exposure. Current estimations of disease risk that are related to *CYP* polymorphisms are premature. However, as determinations of multiple CYP variants with well-characterized functional importance are assessed together, the prognostics are likely to improve. Identifying individuals who are also at a higher risk for drug addiction and disease incidence would provide a strategy for using personalized prevention strategies against the development of drug dependence as well as other chronic diseases.

Therapeutic implications

One clinical reason for characterizing CYP polymorphisms and their influence on addictive behaviors is the potential for identifying novel targets for the treatment of drug dependence. Collectively, the data presented in this review provide evidence that several genetic variations in drug metabolism affect dependence risk. Moreover, mimicking these genetic defects by inhibiting the drug metabolizing enzymes (i.e., CYP inhibition or phenocopying) can modulate drug-taking behavior and experience; this approach has the potential to prevent drug dependence or lead to the cessation or reduction of drug use.

Epidemiological data suggest that between 5–14% of smokers intend to quit in the next year and 2–3% in the next six months, respectively [99] but only very few succeed [100]. Existing treatments for nicotine dependence show poor effi-

cacy compared to treatments for long-term abstinence of other dependencies. Of smokers using aids to quit, only 6–18% will be abstinent at one year compared to smokers on placebo treatments. The health consequences of smoking are due to the harmful components of tobacco smoke, hence reducing smoking can be expected to decrease the risk of lung and other cancers, respiratory symptoms, incidence of chronic obstructive pulmonary disorder, coronary artery disease, peripheral vascular disease as well as complications in pregnancy [101-104]. Exposure reduction may be appropriate for those who fail at cessation before they are ready to try quitting again, are unable to quit (repeat attempts), want to reduce smoking, are highly nicotine-dependent with high long-term risk or have tobacco-related diseases [36,105]. Based on the results of these genetic investigations, CYP2A6 inhibition using the drug methoxsalen was hypothesized to lower the clearance of nicotine and increase its oral bioavailability, thereby reducing the amount smoked by smokers who are also administered an oral nicotine replacement product. As predicted, CYP2A6 activity was significantly inhibited by 30 mg of methoxsalen, which resulted in an increase in plasma nicotine, a decrease in the amount of smoke inhaled and a decrease in the subjects' self-rated current desire to smoke [31,90].

In addition it is known that CYP2A6 can generate NNK hydroxy-metabolites that can alkylate DNA and lead to mutagenesis [106]. CYP2A6 inhibition alone (i.e., in the absence of oral nicotine) in smokers lead to increased excretion of the detoxified NNAL glucuronide presumably by rerouting the mutagenic activation of the procarcinogen NNK to the formation of the detoxified NNAL glucuronide [90]. Since CYP2A6 is not known to metabolize clinically used drugs, except nicotine and SM-12502, inhibition of this enzyme offers a potentially effective and feasible strategy to include in smoking cessation treatments (i.e., oral nicotine pill) and to smoking reduction (i.e., CYP2A6 inhibitor ± nicotine replacement) [37].

The results of genetic and CYP2D6 inhibition studies suggest that reduced CYP2D6 activity differently modulates the effects of addictive drug substrates. EMs may have a greater risk of abuse of dextromethorphan than PMs since EMs experienced positive subjective effects while PMs appear to be at greater risk of adverse effects. The dichotomy of dextromethorphan's subjective effects between EMs and PMs were reflected in the strong correlations between the plasma levels of dextromethorphan or its metabolite dextrorphan with various subjective measures [43]. Hence, 'phenocopying' *CYP2D6* EMs to slower metabolizers, using the potent CYP2D6 inhibitor quinidine, was predicted to modify the experience of an EM individual by increasing the negative subjective drug effects, leading to a reduction in the intake of dextromethorphan and therefore decreasing the likelihood of its abuse. In the case of codeine, genetic studies revealed that PMs were less likely to abuse the drug [44] suggesting that inhibition of CYP2D6 may prove to be a good therapeutic intervention for reducing codeine abuse. A pilot study found that a single dose of quinidine pretreatment not only reduced the level of active metabolites of codeine generated by CYP2D6 but also decreased the positive effects of the drug (e.g., the 'high' feeling) [107]. Moreover, another preliminary investigation showed that CYP2D6 inhibition by fluoxetine significantly decreased opiate use [108]. However, a larger well-controlled, double-blinded investigation [109] failed to reproduce the effect on codeine dependence, suggesting that CYP inhibition for the treatment of codeine dependence is not likely to work, at least in the absence of additional pharmacological or behavioral therapies. *CYP2D6* PMs are protected from developing codeine dependence since they do not experience sufficient positive effects of the drug (metabolite morphine). However, blocking the activation of codeine to morphine in those already codeine dependent appears not to be useful indicating that once dependent, the manipulation of CYP2D6 activity does not alter codeine dependence.

Although little is known about the role of CYP2E1 in the development of ethanol abuse and dependence, there is mounting evidence that CYP2E1 is a key factor in the pathogenesis of alcoholic liver disease [110], a risk factor for hepatocellular carcinoma [111]. Due to the strong correlation between ethanol-induced CYP2E1 and the generation of cytotoxic intermediates, it was proposed that CYP2E1 inhibition may be used for the prevention of alcoholic liver disease. The use of polyunsaturated phosphatidylcholines, which are potent CYP2E1 inhibitors, has been proposed as a preventative strategy for alcoholic liver disease and phosphatidylcholine is currently being tested clinically as an antifibrotic agent in alcoholics [112]. If CYP2E1 induction by various agents, including ethanol and nicotine, is found to contribute

Highlights

- CYP2D6, CYP2A6, CYP2C19 and CYP2E1 are members of the CYP superfamily of heme-based oxidases that are genetically variable and can metabolically detoxify or activate numerous types of drugs, including drugs of abuse.
- Differences in pattern of drug metabolism among individuals and across ethnic groups due to genetically variable CYP isozymes are likely to importantly affect the risk of drug dependence.
- The effects of genetically variable metabolism of drugs of abuse depends on whether the parent drug, metabolite(s) or both are responsible for its addictive properties.
- CYP2A6 is the enzyme that is responsible for the majority of the metabolic inactivation of nicotine, the tobacco smoke constituent that initiates and maintains tobacco dependence.
- Individuals carrying CYP2A6 defective alleles appear to be at lower risk for tobacco dependence and among smokers, slow metabolizers smoke fewer cigarettes compared to those homozygous for wild-type CYP2A6.
- Results of kinetic and psychopharmacological studies indicate that genetic variants of CYP2D6 influence abuse liability of its substrates dextromethorphan, codeine and N-methamphetamine.
- Preliminary analysis of CYP2C19 and CYP2E1 polymorphisms provide evidence that some variants may be functional and could partly explain differences in inter-ethnic sensitivities to the benzodiazipines and ethanol.
- Detecting individuals with genetically altered CYP activity by genotyping or phenotyping to identify those at risk for drug dependence may be an effective strategy for personalized prevention or reduction of drug use.
- Mimicking protective gene defects in CYP2A6 and CYP2D6 using chemical inhibitors provides potential treatment strategies.
- Over the next decade, further research into the impact of genetic variation of these CYPs and their regulators will determine the extent of their impact on drug dependence, possibly leading to identification of at-risk individuals or modulation of these CYPs as novel therapies.

to the abuse of ethanol by increasing ethanol inactivation, the use of CYP2E1 inhibitors might not only reduce the rates of liver disease, but also reduce the risk for ethanol abuse.

Conclusion and outlook

The *in vitro* and *in vivo* pharmacokinetic studies described here provide evidence that genetic variations in drug metabolism have important behavioral consequences that can alter the risk of drug abuse and dependence. However, these studies clearly need to be performed in larger populations of multiple ethnic groups that vary in the frequency of CYP variants and frequencies of drug dependence. Moreover, other CYPs that metabolize drugs of abuse may also be genetically polymorphic and lead to changes in the metabolism of these drugs. For example, recent data has shown that CYP3A4 and CYP3A5, CYP isozymes that biotransform that the majority of drugs, also have genetic variation [113-116]. Furthermore, regulators of CYP expression also exhibit genetic variability. The nuclear receptors aromatic hydrocarbon receptor, pregnenolone X receptor, glucocorticoid receptor and peroxisome proliferator activated receptor, which appear to regulate the expression of various cellular molecules including several CYP isozymes (i.e., CYP1, CYP3A4, CYP2B6, CYP2C9 and CYP4A) may be genetically polymorphic [117-122]. In addition, frameshift mutations have been identified for hepatocyte nuclear factor (HNF)-1α, the nuclear factor that appears to regulate CYP2E1 gene transcription [123,124] and therefore possibly influencing the constitutive CYP2E1 levels. Since there is evidence that some of these variants are functionally different from the wild type alleles, the expression of these nuclear receptor variants may lead to altered constitutive levels of CYP isozymes as well as altered responses to endogenous and exogenous CYP gene inducers and suppressors. Similarly, numerous polymorphisms in other classes of drug-metabolizing enzymes have been shown to affect enzyme function (e.g., N-acetyl transferase, thiopurine methyltransferase and UDP-glucuronosyltransferase) [125,126]. Multiple drug-metabolizing enzymes that alter the pharmacokinetics of drugs of abuse should be investigated simultaneously to improve our understanding of the influence of variation in drug metabolism on the risk of drug dependence and abuse.

From the extensive study of the neurobiological basis of drug dependence [127,128], it is highly likely that variations in biological factors that are part of the neurophysiologic pathways mediating the initiation and maintenance of drug taking are also involved in the risk of drug abuse. Both positive and negative associations of genetic variants in central receptors (e.g., dopamine, nicotinic), enzymes (e.g., for monoamines, tyrosine and tryptophan) and transporters (e.g., for serotonin, dopamine) with the risk for drug abuse have been reported [129]. Clearly, genetic risk for drug abuse is extremely complex. By assessing a combination of multiple genetic variants for which an association with drug abuse has been shown, the likelihood of identifying individuals at higher risk may be enhanced.

The authors have found that mimicking defective CYP genotypes, by chemical inhibition of these CYPs, leads to alterations in the subjective effects of some drugs of abuse. This may reduce the consumption of these drugs in already

dependent individuals or prevent dependence in genetically at-risk individuals that are experimenting with these drugs. Since some of these enzymes can be inhibited or potentially induced without leading to major toxicities with currently prescribed medications, manipulating drug metabolism may provide novel approaches to prevention and treatment for drug dependencies.

Acknowledgements

The authors would like to thank Dr Sharon Miksys and Shari Goodz for review of the manuscript. The authors acknowledge the support of the Centre for Addiction and Mental Health, NIDA grant DA 06889 and CIHR grant MT 14173. Lisa Howard is supported by a CIHR scholarship and Rachel Tyndale is supported by a Canadian Research Chair in Pharmacogenetics.

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